CopeMed II Workshop on methodologies for the identification of stock units in the Alboran Sea

3-6 April 2017
Alicante, Spain
REPORT OF THE COPEMED II WORKSHOP ON METHODOLOGIES FOR THE IDENTIFICATION OF STOCK UNITS IN THE ALBORAN SEA

ALICANTE, SPAIN, 3-6 APRIL 2017
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Preface

The CopeMed II Project on Co-ordination to Support Fisheries Management in the Western and Central Mediterranean is executed by the Food and Agriculture Organization of the United Nations (FAO) and funded by the Government of Spain, represented by the Secretaría General de Pesca (Mº de Agricultura, Pesca, Alimentación y Medio Ambiente, MAPAMA), and the European Union, represented by the European Commission (EC). The premises of the project at the Subdelegación del Gobierno in Málaga (Spain) are part of the Spanish contribution included in the agreement with the FAO.

The objective of the project is to maintain the sustainability of the marine fisheries in the central and western Mediterranean Sea and its ecosystem, taking into consideration environmental, biological, economic, social and institutional issues. In addition, the project will continue to reinforce the collaboration among the participating countries of the sub-region by facilitating their participation in the activities of the Scientific Advisory Committee (SAC) and in the General Fisheries Commission for the Mediterranean (GFCM).

Regions covered by CopeMed II are the western and central sub-regions of the Mediterranean. Participating countries are Algeria, France, Italy, Libya, Malta, Morocco, Tunisia and Spain. The main beneficiaries are the fishery policy-makers, managers and fishery administrations in the western and central Mediterranean countries. The project is also contributing to the strengthening of regional collaboration by supporting the participation of the countries in relevant regional scientific organizations, such as the FAO’s General Fisheries Commission for the Mediterranean (GFCM). Secondary beneficiaries include the national research institutes, fishers and fishers’ associations, and industrial organizations.

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ABSTRACT

A technical workshop for the identification of stock units in the Alborán sea was organised from 3-6 April 2017 in Alicante, Spain. Experts from Algeria, Italy, Morocco Spain and Tunisia presented and discussed current methodologies for the delimitation of stocks with the final aim to design a medium term research program to identify stocks of sardine and hake and their spatial distribution in the Alborán sea (GSAs 1, 2, 3,4 and adjacent waters). Participants agreed on the use of a multidisciplinary approach to stock identification, involving genetics techniques, parasites, life history traits, elemental composition, morphometry and fishery patterns. Sampling of individuals will be designed from the beginning of the study with this purpose, taking advantage of the current surveys in place. A concept note of this research program was developed to be transmitted for further consideration by the Project Coordination Committee in October 2017.
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1. Opening and objectives of the meeting

The meeting was opened by the Director of Casa Mediterráneo, D. Miguel Oliveros who addressed welcoming words to participants. He expressed his satisfaction and honour for hosting this expert meeting convened by COPEMED II project, to discuss about potential methods for the identification of stock units in the Alborán sea. He stressed the functions of Casa Mediterráneo as a public institution that belongs to the Spanish Ministry of Foreign Affairs and Cooperation with the aim to strengthen the active participation of civil society in international relations. Among the subjects that are the competence and aims of the House, he pointed out the environmental matters, and specifically, the environment of the Mediterranean sea. Therefore, the House was pleased to work with the FAO to help in taking any step to improve the sustainability of Mediterranean resources and wished this technical workshop to find the right program to define the stock units of priority species.

The representative of the Spanish donor, Mrs. Encarnación Benito welcomed the participants on behalf of the Secretariat General for Fisheries, belonging the Spanish Ministry for Agriculture and Fisheries, Food and Environment. She thanked the Director of “Casa del Mediterráneo” as facilitator for the organization of the meeting, as well as the team of the FAO COPEMED II Project for all their fruitful work for a successful and productive outcome of the workshop. She also pointed out the important collaboration of the IEO in the organization of the contents of the Agenda, and thanked all the experts attending the meeting for their precious inputs to be made during the following days. Throughout her speech she recalled that Spain had been a pioneer starting the FAO Fisheries cooperation projects with the inception of the first COPEMED Project in 1995 that lasted 10 years, also being the city of Alicante its first Office. The current phase of the Project starting in 2008, COPEMED II, was highlighted to be delivering important outcomes for the region and benefits for the countries involved, as well as increasingly having more relevance in the scientific advice given to the SAC and working groups of the GFCM. On this regard, she raised the importance for fisheries administrations to rely on sound and solid scientific advice to take management decisions, and mentioned the recently adopted “MedFish4Ever Malta Declaration” towards the sustainability of Mediterranean fisheries. Finally, she recalled how the need for this workshop came from the recommendations made at the 39th GFSCM Session and its 38th SAC, and the decision to be convened made at the last COPEMED Coordination Committee held in Malta last year, as there were still many uncertainties regarding the shared character of the stocks in the region of the Alboran Sea. She mentioned the main expected objectives of the workshop as the discussion on potential methods for the identification of stock units in the Alboran Sea, and possibly the design of a research programme to define the stock units for some key species, being the starting point to better understand the possible relationships between these stocks targeted by different fleets in the subregion.
The Copemed Acting coordinator, Mr Marcelo Vasconcellos thanked Casa Mediterráneo and the donors for their support. He highlighted the need for a better sound scientific advice in view of the current situation of overexploitation for the majority of stocks that are assessed annually in the Western sub-region. The establishment of multiannual and multinational management plans being one of the priorities of the GFCM requires a deep knowledge of the stocks boundaries and spatial distribution of the fleets. The current workshop intends to be a first step to match the biological and the managerial limits of exploited resources in an area shared by three countries with relevant fishing industry, the Alboran sea.

Copemed II Fishery Expert, Ms Pilar Hernández introduced the objectives and expected outputs of the meeting: i) Overview of last assessments in Alborán sea and main recommendations by the SCSA concerning the stocks units definition for *Merluccius merluccius* and *Sardina pilchardus*; ii) Overview of existing studies in the sub-region using different methods. iii) Description of hydrogeomorphology and hydrodynamics of Alborán sea and their connection with larval drifting; iv) Revision of other past projects in the Mediterranean like STOCKMED and HOMSIR with the aim to analyse the methodologies and to learn from their experiences and v) overview of existing data and selection of potential method/s for the preparation of a medium term research plan for the identification of stock units in the Alborán sea. The agenda as approved by the group is presented in Appendix I.

Mr Pablo Abaunza, Subdirector of Research of the Spanish Institute of Oceanography was appointed as chairman of the workshop.

2. Overview of current knowledge, historical trends, stock status and uncertainties about stock boundaries of sardine and hake in Alboran Sea

Mr Henri Farrugio, CopeMed consultant, introduced the current knowledge regarding the situation of stocks and fisheries of sardine and hake in the Alboran Sea including the recommendations made by the different technical bodies of the GFCM concerning research priorities for the delimitation of the stocks.

From a management point of view sardine and hake have been historically considered in the Alboran Sea as belonging to 4 independent stocks corresponding to the GFCM Geographical Sub-Areas 01, 03, 02 and 04. These resources are exploited by the Spanish, Moroccan and Algerian fleets and they are still currently managed without consideration of biologically meaningful boundaries of the stocks. their true biological structuring. The effects of fishing pressure on a species in one area on the abundance of the same species in a neighboring area are still unknown and this situation could have an impact on stock assessment and fisheries management advice. Three assessments considering a possible single shared sardine stock in the Alboran sea have been done in 2012, 2015 and 2016; the last advice was that such a stock should be considered sustainably exploited. In 2016 an assessment has been done under a single shared hake stock hypothesis. This analysis showed a very severe status of overexploitation with very low values for the hake biomass and spawning stock. However, in both cases there are still many doubts about these hypotheses and the need to improve the knowledge of the stock boundaries is recognized as a fundamental step for the purposes of assessment and management.
Single technical approaches to improve the stocks identification are often insufficient to delineate stocks and various multidisciplinary approaches are implemented by Regional Fisheries Organizations like ICES, NAFO and ICCAT to define stock boundaries and improve decision-making processes for fisheries sustainability. In the Mediterranean despite its outmost importance, the use of this type of approach is still very rare and any initiative in this sense would be welcome.

Then, on behalf of the Executive Secretary of the GFCM, Mr Farrugio transmitted the following message: “He thanked the Copemed II project for having invited the GFCM to attend this meeting. The GFCM is interested in all the contributions that can help to achieve its responsibilities which are to keep under review the state of the Mediterranean living resources and the level of their exploitation, and also to formulate and recommend, appropriate measures for the conservation and rational management of living marine resources and for the implementation of these recommendations.

The assessments of the state of the resources are performed within geographical sub-areas which delimitation was essentially based on political and statistical considerations without considering the biological structuring of the animal populations.

For several priority species like hake or sardine, the current management measures are not providing tangible effects on the status of the populations. This may be due to the fact that in general the degree of overlap between the current harvest stocks is unknown. As a consequence the stock units can be considered as not well defined and this situation could have an impact on the stock assessment results.

During the sessions of the assessment working groups of the GFCM the importance of delimitation of stocks has been highlighted many times because the scientists where not in a position to determine which were the most valid management units. In particular they asked for an in-depth analysis on stock boundaries and the pertinence of joining GSAs 1 and 3 for sardine in the Alboran Sea.

During its last session in March 2016 in Cyprus, the Scientific and Advisory Committee of the GFCM recommended to further investigate this issue and proposed to compile and analyse information for the identification of stock boundaries as one of the main Subregional issues to carry out in the Western Mediterranean as well as in the Eastern Mediterranean.

In this context the GFCM secretariat welcome the initiative of CopeMed II to design a research program to define stock units in the Alboran Sea, as another responsibility of the GFCM is to encourage research and development activities, including cooperative projects which should produce elements to strengthen its recommendations of scientific based decisions for the management of the marine resources. In addition, he confirmed availability of GFCM to contribute technically and financially to the development and to the execution of all the phases of the research program that is being designed in the current workshop”.

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3. Hydrological characteristics of Alboran Sea in connection with larval drift.

Three presentations were introduced by the experts on this subject. The summary and further discussions are summarized below:

**Hydrodynamic connectivity of the Alboran Sea** *(J. García Lafuente et al. Univ. Málaga-IEO)*

Considering the overall scenario of the general circulation of the Mediterranean Sea, the Alborán Sea is a zonal-oriented basin whose main role is to steer the inflow of Atlantic water through the Strait of Gibraltar towards the interior of the Mediterranean. From this broad point of view its mean surface circulation consists of a west-to-east flow that would isolate the ecosystems of its north and south shores from each other or, at least, establish a considerable obstacle for their connectivity.

This simple, yet realistic, description guarantees the east-west connectivity, which can be extended to the Gulf of Cadiz in the Atlantic side of the Strait of Gibraltar, a source of biological material for the Alborán Sea. Such easily understandable hydrodynamic link is behind the reproductive strategies of keystone fish species of the pelagic domain, which take profit of the prevailing surface circulation pattern to separate spawning and nursery grounds, thus assuring higher survival rates. An archetypical case is the South-African anchovy, whose main spawning ground is at the Agulhas Bank, while the important nursery area is around 500 km further north *(Hutchings et al., 1998)*; in this framework, the species takes benefit of the Benguela current. At a smaller spatial scale, the anchovy inhabiting the northeast coast of the Sicilian Channel deploys a similar strategy, making use of the Atlantic Ionian Stream to differentiate nursery and spawning areas *(García-Lafuente et al., 2002)*. In all these cases, the connectivity of the areas is warranted by the local circulation that displaces the spawning products downstream with no obstacles. However, cross-stream transport is a much more difficult task.

Coming back to the Alborán Sea with a bit more detail, the aforementioned eastward flow performs like a pronounced meandering stream that, as a rule, encircles two medium-size anticyclonic gyres where newly arrived Atlantic water accumulates at their centers. The meandering jet heads to the south when it goes around the eastern edge of the gyres, so it has clear potential for north-south transport of biological products. Despite this, the jet still represents a physical barrier and the possibility of this material arriving finally at the south shore’s ecosystem depends on its capability to go across the jet, which is not easy for passive tracers or nektonic organisms. Physically, it is the prevailing positive vorticity in the left side of the jet (looking downstream) that would prevent the across-stream ageostrophic transport and would keep the waters of each side of the jet in their own side.

A more detailed analysis, however, confirms that this jet has instabilities which break apart the smooth hydrodynamics sketched above. The anticyclonic gyres and the encircling jet are not permanent structures. At sub-inertial (weeks to months) and seasonal time-scales, either one or other gyre or both them may disappear and then reform again. It changes noticeably or, even, dramatically the surface circulation of the Alborán Sea and breaks momentarily the dynamic barrier the jet represents. Such situations have been scarcely documented by in situ observations. One of them, analyzed in García-Lafuente et al. *(1998)*, occurred in summer of year 1993. Numerical simulations, however, suggest that these processes are not infrequent. In a recent study, Sánchez-Garrido et al. *(2013)* show that the instabilities are not sporadic processes but rather they are the time evolution of structures that are permanently fed up by the energetic Atlantic jet entering through the Strait of Gibraltar. This jet also contributes to
feed a small and rather permanent cyclonic gyre located off the Spanish shore (cf. Estepona for an easy visualization) with positive vorticity. Interestingly, this is an upwelling area that gathers suitable characteristics for small pelagic spawning. Under favorable forcing mechanism such as propitious meteorological conditions or strong – spring- tides, the cumulated positive vorticity enlarges the cyclonic eddy which may trigger instabilities that can end up with the collapse of the Western Alborán gyre. The cyclonic eddies, which enclose water and products from the northern part of the Alborán Sea, are then released from its source place and propagate along the outer rim of the Atlantic Jet without disturbing the gyre. There are chances that they end up in the southern shore of the Alborán Sea, thereby establishing an intermittent connection between both shores. But this intermittent transport does not guarantee the survival of larvae unless larval trophic resources are made available during the drift passage.

Explicit implementation of connectivity estimates in population dynamics and assessment frameworks: the European hake in the northwestern Mediterranean (J.M. Hidalgo. IEO)

Worldwide overexploitation of fish populations challenges emerging solutions that incorporate ecosystem and environmental complexity into modeling and assessment stewardships. Predicting marine fish production from physics is an important contemporary demand. However, fisheries assessment of large populations is in general based on deterministic models that lack realistic information from important stochastic physical processes despite their expected crucial importance to reproduce properly population dynamics trajectories. The new generation of stock assessment models will come through the improvement in two axes: a) a better implementation of the structural complexity of populations and b) a wise parameterization and explicit integration in a simple and straightforward manner of those ecological processes that are spatially structured.

The case of European hake, has been shown to exemplify how we can model recruitment dynamics in terms of physics-dependent drivers: larval dispersion and retention, and climate influence on early life stages survival. The results shown evidence that fish recruitment in the Mediterranean can be largely reproduced incorporating connectivity estimates derived from ocean circulation models and the spatial dependence of hydroclimatic influence. This study opens new and broad opportunities to improve fisheries management by including short-term projections of physical oceanography, particularly in populations with complex spatial structure.

Connectivity between spawning and nursery areas. What is the right spatial scale for our stock assessment? (F. Fiorentino. IAMC-CNR)

Stock assessment is a complex discipline in which main sub-models dealing with description of fishery resource dynamics are classically combined in a “black box” where the spatial dimension of the population and exploitation characteristics are not considered. However it is well known that exploited populations are spatially structured. Four main type of spatial structures in marine populations are known: i) unit population with spatial structure, where individuals of the species are unevenly distributed within a single reproductive population; ii) spatial isolated population where organisms form a single, self-sustaining biological unit that does not overlap in space with other populations; iii) natal homing where individuals maintain self-sustaining populations through natal homing despite spatial overlap with other populations during certain time;
iv) meta-populations where multiple, mainly self-sustaining, subpopulations are connected through migration between units, while organisms do not demonstrate spawning site fidelity.

After having outlined the importance of stock recruitment relationships in ensuring renewability of exploited population, the main aspects to be considered in constructing a simulation model of complex population structure for stock assessment and management purposes were briefly discussed. In particular results obtained in a recent study on the connectivity between spawning and nursery areas of red mullet (Mullus barbatus, L. 1758) in the Strait of Sicily (Central Mediterranean) based on dispersal model are depicted (Gargano et al., 2017). The numerical simulations revealed a certain degree of connectivity between the Sicilian–Maltese and the African sides of the Strait of Sicily. Connectivity is present in both directions, but it is stronger from the Sicilian–Maltese spawning areas to the African nurseries owing to the marine circulation features of the region. However, the number of individuals recruited on the northern side of the Strait which are born off the southern coast and vice versa can be considered as negligible. Furthermore, because the majority of the larvae are transported to areas unsuitable for settlement or outside the Strait of Sicily, the dispersal process is characterized by a strong loss of potential settlers born in the spawning areas. These results are in agreement with the low genetic heterogeneity reported for this species in the Mediterranean Sea and support the existence of a meta-population structure of red mullet in the SoS and the adjacent areas. Furthermore findings strongly support the current procedures adopted in the STECF and SAC working groups to assess separately stock status of red mullet in northern and southern side of the Strait of Sicily.

Finally some questions linked to the connectivity between source and sink habitats in spatially structured populations to be considered in stock assessment and sustainable exploitation were presented and discussed. Three main aspects were outlined: i) what is the fraction of the recruits we observe which are born in the study area and what that originated in the adjacent seas; ii) how we can contribute to reach the MSY protecting nurseries (increasing the size at first capture to have a better value of Y/R at a given level of fishing mortality/effort), considering the migration of juveniles from nurseries to feeding or spawning areas; and iii) how we can contribute to reach the MSY protecting spawning areas (increasing the number of recruits by a SSRR approach) within the area under study, considering the larval drifting and settlement.

4. Current approaches and methods used to define stock boundaries.

4.1. Genetic markers

General considerations for a genetic approach to the identification of stock units in the Alboran Sea. (C. Johnstone and M. Pérez. IEO)

Genetics can provide useful tools for different aspects of fisheries, for the understanding of harvested species, measuring the impact of fishing, as a tool for biosecurity or for product surveillance (Ovenden et al., 2015). In order to discuss genetics as a potential method for the identification of stock units in the Alboran Sea, it is important to consider the type of genetic markers in addition to the type of specimens to be analyzed in terms of number, age, sampling strategy among other aspects. Allozymes, restriction fragment length polymorphisms (RFLPs), DNA sequences, expressed sequence tags (ESTs), random amplified polymorphic DNA (RAPD), single nucleotide polymorphisms (SNPs), microsatellites or AFLPs (amplified fragment length
polymorphisms) are some of the molecular tools available for population genetics (Freeland et al., 2011). In general, stock boundaries have been assessed by establishing genetic diversity of populations through mitochondrial or nuclear markers. Partial or complete sequences of a given gene encoded in the mitochondrial genome (mitochondrial markers) have been used to assess species and populations but however have limitations as they represent a single locus and can be affected by hybridization events between species. Analyzing multiple markers in the nuclear genome (nuclear markers) such as microsatellites or SNPs overcomes such limitations.

In addition, genetic variation itself can be neutral or adaptive if it is influenced by environmental change. Neutral markers are the best choice to investigate gene flow, migration or dispersal, which means connectivity. Non-neutral markers are informative in terms of the adaptive or evolutionary potential of a certain species or population. The use of genetic tools for marine fisheries has been recently reviewed for the 2004-2014 period in which microsatellites were the most used marker (Cuéllar-Pinzón et al., 2016). Currently, there are two types of markers that are being used in the majority of works in order to decipher stock identity. These markers are microsatellites as neutral and SNPs as non-neutral. A cost-effective design for a research program to genetically define stock boundaries of priority species fished in both sides of the Alboran sea could be a nuclear multilocus approach combining neutral and non-neutral microsatellite markers, analyzing adults as well as larvae and juveniles to ensure correct stock origin assignment of specimens collected at spatial and temporal compatible scales.

**Population genetics of hake species along the Moroccan coasts for fishery management purposes.** (K. Mokhtar-Jamaï. INRH)

Hake fishery account for 12% of demersal fisheries in Morocco with three exploited hake species: *Merluccius merluccius*, *Merluccius senegalensis* and *Merluccius polli*. During the last years, a decline in European hake landings has been observed reducing catches to half between 2003 and 2013. Therefore, for fishery management purposes, a population genetics study of *Merluccius merluccius*, *Merluccius senegalensis* and *Merluccius polli* using microsatellite markers is currently underway at the Institut National de Recherche Halieutique (INRH) in Morocco, in order to delimit stock boundaries within Moroccan waters and between Morocco and Mauritania for *M. senegalensis* and *M. polli*. For *Merluccius merluccius*, a total of 15 locations will be sampled from Nador (in the Mediterranean Sea) to Dakhla in south Morocco (in the Atlantic Ocean) and 600 specimens will be screened at 12 already developed microsatellite loci. For *Merluccius senegalensis* and *Merluccius polli* specimens will be sampled in the Atlantic Ocean from Agadir (in Morocco) to Cap Roxo (in Senegal). Microsatellite loci will be developed for both species.

**Genomic approaches and targeted genetic markers can identify fine-scale structuring in marine fish populations.** (A. Cariani and F. Tinti. GenoDREAM-BIGeA)

The correct delineation of the different biological units of any exploited fishery resources is an essential and critical background for proper implementation of knowledge bio-based management and conservation measures.

In the last two decades, the development of genetic techniques allowed the refinement of the ecological “stock concept” to include a degree of reproductive isolation. Molecular genetic techniques and neutral genetic markers can identify reproductive
isolation between populations, permitting the identification of biological units and the verification of their correspondence to the management units (i.e. fishery stock).

However, shallow weak signal of population structure are generally reported for most marine fish using neutral genetic markers and explained as a consequence of high dispersal, connectivity and large population size. More recently, genome-wide studies and targeted analysis of markers linked to functional gene regions have challenged such view, suggesting that divergence might occur even when neutral markers provide genetic homogeneity across populations and revealing patterns of fine-scale genetic structuring in marine species.

Significant correlation of allele frequencies at specific loci with seawater surface temperature, salinity and other environmental proxy supports the hypothesis that marine populations might be adapted to local conditions (i.e. hake and sole in the Mediterranean). Local adaptation suggested by specific loci is often found to be in a delicate balance with gene flow in marine species with high dispersal potential. Such findings highlight the importance of integrating information from multiple and independent disciplines towards a better assessment of the patterns of population and stock structure, in order to develop explicit spatial models for defining management units and stock boundaries.

4.2. Morphometry and meristics.

Analysis of morphometric variability in Chelidonichys lucerna populations (triglidae) from Algerian coasts. (T. Filali. CNRDPA)

This biometrical investigation was performed on populations of Chelidonichys lucerna collected in different fisheries of Algerian coast. We are selected thirty-one morphometric characters to answer the question of intraspecific morphological variations observed in anterior study. The principal component analysis (PCA) showed the isolation of three populations found within geographical origins. The first specific to the Western region, the second, stretching from Tenes to Bejaia (Central region) and the last specific to the Eastern region of the Algerian coast. This observation, which is essential in the study of stock’s exploitation, should be confirmed by a genetic study.

Discrimination between stocks of sardine in Algerian coast using the number of vertebrae. Bibliography analysis. (K. Ferhani. CNRDA)

It is important to know if sardine (Sardina pilchardus) from the Algerian coast belongs to one or more stocks. Trying to answer this question, some authors selected a single meristic character namely the number of vertebrae.

Bouaziz et al.2014. The comparison of the vertebral mean of sardine from central region (m = 51.21 ± 0.08, n = 228) with those captured in Arzew in the Western part (m = 50.7 ± 0.14, n = 90) and Skikda in the Eastern part (m = 51.55 ± 0.07, n = 323) revealed a gradual but statistically significant increase of the mean value from West to East.

Djabali et al.1992. This study identified two groups of sardine along the Algerian coast: the first group off the western sector with a vertebral mean of 51.29 and the second one off the central and eastern regions with mean of 51.41.
Biological parameters of small pelagic fish as an indicator of variability (Case of the Moroccan Atlantic). (F. Wahbi.INRH)

The stocks identity is very important for the evaluation of resources and for management purposes. The biological characteristics of the species contribute, among other things, to the delimitation of these stock units.

In Morocco, the fishery for small pelagics constitutes the major part of the fishery potential of the Moroccan EEZ. It accounts for 80% of the national production with a dominance of the sardine, most of which is caught off the Atlantic coast.

Long-term conservation and rational management of these stocks is based on scientific advice through regular monitoring of several parameters related to species (biology, structures, abundances, state of exploitation, etc.). A good knowledge of these biological parameters is a prerequisite as inputs for evaluation models and contributes to the identification of fish stocks.

Since the 50s, morphometric studies of sardines have contributed to the adoption of 4 stock units: Stock North, Stock A, Stock B and Stock South C. Subsequently 3 units were adopted (80s) at the base of commercial fleet and acoustic surveys observations which showed migration of sardine from stock A to B and therefore an assessment of stocks A and B as a single stock. Recent more detailed biological studies, of the metric and meristic parameters confirm the phenotypic separation between the central and southern stocks.

Given the importance of these parameters for the stocks identity, studies in progress concern other species such as chub mackerel and Atlantic bonito. Similarly, depending on the transboundary character of certain species, the regional context is highly recommended for this type of study with standardization of sampling methods for processing and analysis.

4.3. Life history traits (growth, reproduction, length-at-age, body condition)

State of knowledge on fisheries of sardine and hake in the Moroccan Mediterranean sea. (M H. Idrissi.INRH)

Five main ports in the Moroccan Mediterranean are operational. In 2015, about 113 purse seine vessels were active with 10,000 sea trips and 43 trawlers with approximately 3100 sea trips.

The average technical characteristics of coastal purse seine vessels are modest (boat size= 19m, TJB= 50tx and motor power= 252 horse power). The fishing area frequented by the purse seiners extends over the entire Mediterranean area but we can delimitate 4 fishing areas. The distance from the coast usually varies between 1 and 8 nautical miles and the depth of the areas frequented generally between 45 and 80 meters.

The activity of purse seiners decreases from February to June due to climatic hazards and the migration of some purse seiners to the Atlantic Moroccan ocean.

Regarding catches, sardine is the dominant species (75%). The annual evolution of small pelagic catches since 2008 shows fluctuations from one year to the next with a maximum in 2009 (14000 tons) and a minimum in 2011 (9000 tons).

The sampling program consists of 3 components: biological sampling of landings, sampling aboard commercial vessels and acoustic surveys. The echo survey covers all
the continental shelf ranging between isobaths 20 and 400 m of depth. Acoustic surveys provide map of the spatial distribution of densities by species. The criteria to be analyzed for stock identification are as follows:

- Size at maturity (available in 1999-2002 and 2013-2015)
- Size frequency in landings (2000-2015)

The size structure of the sardine shows two modes. A main mode at 13cm and a secondary mode at 16.5cm. The monthly evolution of the RGS shows that the spawning peak is observed between January and March. Recruitment takes place between May and August. The parameters of Von Bertalanffy of sardine in 2015 are: Linf= 21cm ; K= 0.71 ; t0= -0.55 an. The length-weight relationship parameters: a= 0.0071 and b=3.038.

For hake, the catch series are available since 1990, the fishing effort series since 2003 and the CPUE series since 2003. The demersal surveys provide distribution maps and hake abundance index on the Mediterranean coast.

The size structure of hake in 2015 shows a single mode located at 22cm. Spawning period is localized in winter like sardine. The size of first maturity in females is located at 34.7 cm.

The criteria to be analyzed for stock identification are as follows:

- Size at maturity (available in 2015)
- Spawning period (2015)
- Sex-ratio (2006-2011 and 2015)
- GSI and its annual dynamics ((2006-2011 and 2015)
- Size frequency in surveys (2006-2015)
- Size frequency in landings (2006-2011 and 2015)

Biological indicators are very important in the delimitation of stocks. We hope that through this workshop, we can develop research actions such as morphometric and meristic analysis that do not cost very much but bring more information to the biological data.

**Contribution to the identification of the stock structure of *Sardina pilchardus* along the Tunisian coasts using growth patterns and body shape (A. Gaamour)**

Along the Tunisian coasts, sardines taken from commercial and experimental catches show large variation in size across coastal and offshore waters. These differences in size may reflect size-specific migrations between areas (Nøttestad *et al.*, 1999) or alternatively separate growth patterns (Borsa, 2002, Gaamour and Khemiri 2010) that can be used to define or corroborate stock structure.
First, we investigated variation in sardine growth from northern (deep-sea water) and southern (wide shallow area) areas along Tunisian coasts. In the two areas, sardines showed a positive allometry. However, at the same length, sardines of the off-shore area (north area) were heavier than those of the coastal water (south area). In both areas, the sardines have short life-spans and grow fast. In the same area, no growth difference was recorded between males and females. However difference in growth pattern between the two areas was statistically significant, having the sardines from the North a size at age a larger than those from the South area.

Moreover, a morphometric analysis of the body shape of sardines caught from inshore area and from offshore area of the gulf of Gabes showed that Sardine from inshore area seems to be flatter than those from open sea which were more fusiform.

4.4. Growth marks and elemental composition of otoliths.

**Contribution to the identification of the stock structure of Sardina pilchardus along the Tunisian coasts, using otolith shape and microchemistry analysis. (S. Khemiri. INSTM)**

*Sardina pilchardus* is of great economic importance in Tunisia. However, little is known its stock structure. Otolith shape and microchemistry analysis were used to discriminate populations of sardine in the open sea and the coastal area of Tunisia. The chemical composition of whole otoliths was analyzed using solution-based inductively coupled plasma mass spectrometry (ICPMS). Linear discriminant analysis classified fish into open sea and inshore groups with close to 94% accuracy for the sardine and to 97% for the anchovy. Respectively, the discrimination was based on P, Sr and B for the sardine and P, Fe, Zn, Sc andSr for the anchovy. In addition, when we pool together the two species data in each area (inshore/open sea), the discrimination was also accurate reaching 88% of well classification based on P, Fe, Li and Mg. The results reveal a clear discreteness of the open sea and inshore small pelagic groups.

Moreover, we investigated the stock structure of sardine by using otolith shape analysis. This latter was determined using Fourier analysis and compared among specimens sampled from different areas with forward stepwise canonical discriminant analysis. We found significant differences in otolith shape between open sea and inshore sardine groups. Such information will have major implications for fisheries management of sardine in Tunisia.

**Applications of stable isotope analysis for fish regional stock, spatial segregation and ecosystems connectivity within the Alboran Sea. (R. Laiz-carrión et al. IEO)**

Isotopes are forms of the same element that differ in the number of neutrons in the nucleus and often have skewed distributions on Earth, mostly reflecting details of their synthesis long ago in stars. Food web tracers, such a stable isotopes of carbon and nitrogen (δ13C and δ15N) could provide intrinsic tags to study the foraging and migratory ecology of marine species. Stable nitrogen isotopes become enriched at successive trophic levels, as more of the light isotope (14N) is secreted than the heavy isotope, living the animal enriched in15N relative to its food source. Stable carbon isotopes can indicate feeding and carbon flow because there is limited fractionation from prey to predator, and different energy sources can have distinct δ13C values.
Stable Isotopes analysis (SIA) can be used to reconstruct the movement and dietary histories of animals. This approach relies on spatial variations in the abundances of ambient isotope or elemental ratios that are recorded in the chemical composition of tissues as an animal lives and feeds in different habitats with distinctive isotopic signatures. An animal’s isotopic composition can be used as a natural “tag” to track their movements through isotopically distinct habitats (isoscapes) (Hobson 1999; West et al. 2010; Graham et al., 2010; Olson et al., 2010).

Stable isotopes are useful tools to investigate ecological segregation within and among species of fish, and can give insights into mechanisms of ecological segregation in diet and spatial distribution. The use of intrinsic markers such as naturally occurring stable isotope ratios of light elements that can be linked to spatial patterns within food webs requires only a single capture of individuals and so can provide an unbiased estimate of origin.

Requirements and advantages of bulk-SAI and compound-specific stable isotope analysis (CSIA) of amino acids in consumer tissues where discussed. In CSIA, predator tissues alone are adequate for trophic-position estimates, and separate analysis of the isotopic composition of the base of the food web is not necessary. (McFarlane 1990; McClelland and Montoya, 2002; Popp et al., 2007; Chikaraishi et al., 2009; Hannides et al., 2009; McMahon et al., 2010; Uriarte et al., 2016)

The stable isotope composition of an organism’s tissues is a reflection of its diet and environment. Because otoliths are continuously growing, are acellular and therefore metabolically inert after deposition, they have the potential to preserve chemical signals specific to particular periods that reflects the diet of the organism at the time of tissue synthesis, providing a continuous time series of diet and environment (Thorrold et al., 1997). Several studies have shown that stable oxygen ratios in otoliths can be used as a proxy of the ambient sea temperature, with lower values indicating higher temperatures and lower salinity (Hidalgo et al., 2008). Otolith stable carbon isotope ratios are also influenced by fish metabolism and diet and isotope ratios of the dissolved inorganic carbon in the water (Javor and Dorval 2014). The relative contribution of these various factors to otolith carbon isotope ratios is likely to vary both in space and in time, and among species. Both isotope ratios have been used successfully as natural tags for fish stock discrimination studies (Thorrold et al., 201; McMahon et al., 2011; Rooker et al., 2014; Fraile et al., 2016).

Stable isotopes in fish can record trophic dynamics in an integrated way, and some examples in sardine, anchovy, hake and tunas early life stages will be discussed as an approach to study stocks differentiation in Alboran Sea.

4.5. Parasites as natural markers.

Integrating parasites as biomarkers in the frame of a holistic approach for stock identification of pelagic and demersal Mediterranean Sea fish species. (S. Mattiucci. Sapienza - University of Rome)

The unique environment of the Mediterranean Sea makes fish stock assessment a major challenge. Stock identification of Mediterranean fisheries has been based mostly from data on biology, life-history characteristics, morphometrics, fish population genetics, artificial tags, otolith shape and microchemistry, and, only in recent years, with an
holistic approach (Begg and Waldman, 1999, *Fish. Res.* 43:35–44) also including the use of parasites as "biomarkers" (for a Review: Mattiucci et al., 2015, *Parasitology*, 142:90-108). The parasite based methodology - including parasite community structure of the fish species along its geographical distribution, parasitic infection levels and genetic/molecular characterization of parasite species - represents an important approach in defining a fish stock (Timi & MacKenzie, 2014, *Parasitology*, 142:1-4). Indeed, parasites can provide ecological information on the origin, migration, nursery ground, life history of the fish species. Basically, a parasite can be used as a suitable biological tag for fish stock identification when its geographical distribution and life cycle are known, and when the parasite’s residence time in the host is long enough, compared with the lifespan of the fish host. As a consequence, the fish host population become infected with a parasite species only when it is spending part of its life-history in the endemic area of that parasite species, namely that geographical region in which the conditions are suitable for the transmission (MacKenzie, 2002, *Parasitology*, 124, 153–163). Furthermore, for parasites having direct life-cycle, the endemic area is constituted by environmental parameters, in the case of parasites having indirect life-cycles, suitable hosts and their density must be present to ensure parasite transmission to that fish host species. Therefore, although not always available, the knowledge about the oceanographic and biological characteristics of a basin water from where the fish population understudied have been sampled, are necessary to interpret resulting data sets to use parasites as fish hosts biomarkers. Additionally, some characteristics should be taken into consideration to be matched, when a parasite species has to be selected as a "biomarker" in fish stock identification. It should be: 1) preferably endoparasites, rather than ectoparasites; 2) not highly pathogen to the fish species; 3) identified at the species level; 4) specialist to that fish species and present at high prevalence. Among the parasite species that matched those requirements and which have been mostly used in the literature, as "biological tags" in fish stocks identification, there are the larval stages of anisakid nematodes of the genus *Anisakis* (Mattiucci et al., 2014, *J Parasitol*, 100:199–214). These nematodes are heteroxenous parasites involving marine mammals (mainly cetaceans) as definitive hosts, while crustaceans (krill), fish and squid act as intermediate/paratenic hosts in their life cycles (Mattiucci & Nascetti, 2008, *Adv. Parasitol.*, 66: 47–148). Nine species have so far been detected by means of molecular/genetic markers, as belonging to the genus *Anisakis* (Mattiucci & Nascetti, 2008 cit. ref.; Mattiucci et al., 2009 *Syst. Parasitol.* 74: 199–217; Mattiucci et al. 2014, cit. ref.); they have shown different genetic structure and ecology (including host preferences and geographical distribution).

Here a review of the research carried out on the use of larval parasites of the genus *Anisakis*, as biological markers, to study the stock structure of some demersal (European hake, *Merluccius merluccius*), and pelagic (horse-mackerel *Trachurus trachurus*) fish species in the Mediterranean Sea, are briefly presented. Specifically, it focuses on the research carried out, on the simultaneous use of differential distribution of *Anisakis* spp. larvae, genetically recognized, their population genetic structure, and their infection levels, as *biomarkers* to assess sub-population structuring of pelagic and demersal fish species in the Mediterranean Sea, also in comparison with populations of the same fish species from the Atlantic Ocean.

Results in the existence of different stocks of a fish species from the use of statistical differences in the distribution and infection levels of parasites (specifically anisakid nematodes of genus *Anisakis*) as biological tags in some fish species are here reviewed.
The relative proportions of larvæ of different species of *Anisakis* in different sampling localities of *M. merluccius* allowed the recognition of Mediterranean and Atlantic stocks of European hake (Mattiucci *et al.* 2004, 65, 495–510). *Anisakis pegreffii* was identified in all the hakes sampled from the Mediterranean Sea, except in the Levantine Sea (off the Cyprus coast). In the western Mediterranean Sea, *A. pegreffii* was the most prevalent and abundant species in the Ligurian Sea. This species also occurred at high prevalence and density in fish sampled from the eastern part of the Mediterranean Sea, such as in the Ionian, Aegean and Cretan Seas. Interestingly, hakes from Levantine Sea waters harboured only the species *A. typica*, a species parasitic in many cetacean species in warm temperatures and tropical waters (Mattiucci & Nascetti, 2008, cit. ref.). *A. physeteris* was the most prevalent species in hakes from the western Mediterranean Sea off the Balearic Islands, Alicante and Malaga (Mattiucci *et al.* 2004, *J Fish Biol*, 65, 495–510.). However, to date hakes from the western Mediterranean Sea, were not found infected with *A. simplex* (s.s.), although this parasite species has been documented in some pelagic fish species, such as mackerel *Scomber scombrus* and horse mackerel *T. trachurus* from some of the collecting areas. While, interestingly, few larvae of *A. simplex* (s. s.) co-infecting the same individual fish with *A. pegreffii* were detected in hakes sampled from the southern part of Alboran Sea (Cipriani *et al.*, *Fish Res.* under review). Conversely, in the North-East Atlantic hake samples from north of the Strait of Gibraltar, *A. simplex* (s.s.) was the most prevalent species, while it occurred off the Spanish Atlantic coast in sympatry with *A. pegreffii*. Indeed, hakes that exhibited mixed infections of *A. simplex* (s.s.) and *A. pegreffii* represented >20% of the fish examined from the Atlantic coast of Galicia and 14% from the Bay of Biscay (Mattiucci *et al.*, 2015 cit. ref.). Moving south in the North-East Atlantic from the Strait of Gibraltar, mixed infections of different species of *Anisakis* in hakes caught along the Atlantic coast of Morocco were observed. More than 22% of the fish examined were found to be parasitized by five species of *Anisakis*: the major component species was *A. pegreffii*, followed by *A. physeteris*, *A. ziphidarum*, and, at a lower percentage, *A. brevispiculata* and *A. paggiae*.

The overall finding supports the hypothesis that there are no migrations of *M. merluccius* from and into Atlantic waters. Thus, according to the pattern of distribution of larval *Anisakis* spp. and their parasitic abundance levels in the hake samples, different populations of *M. merluccius* in European waters were identified. The larval distribution and abundance of the different species of *Anisakis* recognized in hakes from the different fishing grounds indicate that: (1) there are two stocks of *M. merluccius*, from Mediterranean and Atlantic waters respectively; (2) in the North Atlantic area, at least two distinct subpopulations are present, one north of the Strait of Gibraltar and another from off the Atlantic coast of Morocco; (3) some substructuring of the western and eastern parts of the Mediterranean hake populations seems to be recognized, with the population from Levantine Sea distinct from the other Mediterranean ones (Mattiucci *et al.* 2015, cit. ref.). Interestingly, in the Principal Component Analysis (PCA), the sample from the Alboran Sea resulted to be more similar to Atlantic populations. The parasitological findings were found in agreement with the bulk of biological data, showing separate Mediterranean and Atlantic stocks of European hake since they are known to live in markedly different environments and to differ in many biological and demographic features such as growth rate, spawning season and recruitment. Further, they were largely in accordance with the genetic results obtained by using different molecular nuclear markers (allozymes, microsatellites, SNPs), showing a main subdivision between Atlantic and Mediterranean stocks (Cimmaruta *et al.* 2005; Mol Ecol 14:2577–2591; Milano *et al.* 2014, *Mol. Ecol*. 23:118–135, and ref.
therein). The molecular markers indicated that the boundary between the two stocks is located along the Almeria-Oran Front (AOF), instead of the Strait of Gibraltar. The high agreement among the different datasets in identifying the subpopulations of European hake was supplemented with a generalized Procrustes Rotation, which compared different ordinations of the European hake specimens as inferred from the combination of the genetic analysis (data from Cimmaruta et al. 2005 cit. ref.) with the larval distribution of the different *Anisakis* species, as detected in the same subpopulations.

Of the five species of *Anisakis* identified in horse mackerel, *A. pegreffii* and *A. simplex* (s.s.) were found to be the dominant species in all the fish sampled along its geographic distribution, whereas the other three species, i.e. *A. physeteris*, *A. typica* and *A. nascettii*, were identified at very low percentages in the central Tyrrhenian Sea, the Cretan Sea and in the NE Atlantic fish samples. *Anisakis pegreffii* and *A. simplex* (s.s.) showed statistically significant differences in their relative proportions in the horse mackerel samples throughout the NE Atlantic and the Mediterranean Sea. Indeed, *A. pegreffii* was identified as the main species parasitizing horse mackerel in the Mediterranean Sea. Mattiucci et al. (2008 Fish. Res. 89, 146–151) found that the samples of horse mackerel fished in the Alboran Sea showed mixed infections by both the sibling species, *A. pegreffii* and *A. simplex* (s. s.) in almost equal proportions. Moving to the NE Atlantic Sea, the occurrence of *A. pegreffii* progressively decreased from almost 87% off the Portuguese coast of Algarve, to 30% in the horse mackerels from off the Spanish Galician coast. *Anisakis pegreffii* was also rarely identified. Finally, parasites and morphometric analyses were congruent in depicting the existence of sub-structured areas of horse mackerel in the Mediterranean Sea (western, central and eastern) (Abauanza et al. 2008, *Fish. Res.* 89:196–209). The Generalized Procrustes Rotation (PR) comparing different ordinations of the horse mackerel populations based on combined molecular and parasitic burden datasets showed slightly significant correlation between the parasitic burden and molecular traits gathered on the same fish species. This finding further supports the idea that parasites show structuring of their fish host populations at regional and temporal scales. Indeed, the latitudinal patterns of the larval *Anisakis* spp. distribution delineated the horse mackerel putative stocks in the Mediterranean and Atlantic waters according to the different regions of basin waters inhabited by different host meta-populations of the pelagic fish species, i.e. the Mediterranean and Atlantic horse mackerel (Mattiucci et al., 2015 cit ref.).

Finally, the possible use of the phylogeographic analysis of a parasite species, selected as a biomarker, and collected from different fish meta-populations, has been recently suggested (Baldwin et al. 2011, *J Parasitol*, 97: 545-554; Klapper et al., 2016, *Plos One*, 11, 4.; Mattiucci et al., 2017 *Fish Res.* under review), as a further tool to be included in multidisciplinary studies on fish stock structure. Specifically, preliminary data on the population genetic structure inferred from the phylogenetic analysis of mtDNA cox2 gene locus was carried in populations of *A. pegreffii* sampled from *M. merluccius* from eastern, western Mediterranean Sea waters, in comparison with the Atlantic populations of the same species of *M. merluccius* (Mattiucci et al., data not published). Population genetic differentiation of *A. pegreffii* among the different fishing areas was estimated, at the intraspecific level, based on mtDNA cox2 sequences. Spatial comparison based on molecular variance analysis and *F*ₐ values was performed for the collected specimens (among regions). Haplotype network construction showed relevant differences in haplotype frequencies between samples of *A. pegreffii* from the different geographical areas. Results indicate a genetic sub-structuring of *A. pegreffii* obtained from hakes from different basin waters, with the population from the Atlantic Ocean
(Spanish-Iberian coast) being the most differentiated one, with respect to the those from hakes populations of the eastern (Adriatic Sea) and western Mediterranean Sea (Tyrrhenian Sea) basin waters (Mattiucci et al., data not published). It appears that the population genetic structure of A. pegreffii was in accordance with the hakes population genetic structure throughout the host’s geographical range. Finally, results suggest that mtDNA cox2 is a suitable molecular marker for population structure analysis of these parasites species. Additionally, host-parasites co-phylodogeography would represent a further analysis to be included, in a holistic approach, in defining fish stock structure from different basin waters.

Spatial variability of metazoan parasites and evidence for stock discrimination in small pelagic fishes off the coast of Tunisia (M. Feki. Faculty of Sciences Sfax)

Small pelagic fishes have great commercial importance in Tunisia and reported landings of about 52,451 tons in 2011. Catches of each species have undergone fluctuations during this decade in various areas of Tunisian coasts. An accurate knowledge of the number of fish stocks is a basic requirement to ensure a sustainable fishing. Using parasites as biological tags to provide information on the stock discreteness of their fish hosts is an established methodology, with broad acceptance as a tool for helping to resolve fishery management problems. A total of 1329 individuals of three of pelagic species Sardinella aurita, Scomber colias and Trachurus trachurus were collected at 5 distant fishing localities off Tunisia: Bizerte, Kelibia, Mahdia, Gabes and Zarzis. Twenty three parasites species were recorded and infection parameters were calculated and their infestation parameters were monitored over a period of one year. Non-parametric tests and discriminant analysis were used to analyze differences in prevalence and mean abundance of parasites in these fish. Indeed, four selected parasites allowed for the identification of two stocks of S. aurita. Specimens of S. colias and T. trachurus were separated in three stocks using respectively nine and ten parasites species. One stock in the north from Bizerte, one in the gulf of Hammamet from Kelibia and Mahdia and one in the south from Zarzis. Selected parasites from the three pelagic fishes are good biological tags in stock discrimination off the coast of Tunisia.

Two stocks of S. aurita were identified, one in offshore waters from Bizerte, Zarzis and Kelibia and one in inshore waters from Mahdia and Gabes. Specimens of first stock were characterized by a very high level of parasitism for Parahemiurus merus and Aphanurus stossichii. Individuals from the second stock were characterized by a high prevalence of Tetraphyllidea larvae.

Atlantic chub Mackerels S. colias were separated in three stocks; one at Bizerte characterized by the absence of Grubea cochlear, Kuhnia scombri and Pseudokuhnia minor, one regrouping specimens from Kelibia and Mahdia characterized by Prodistomum orientalis and one at Zarzis correlated positively with G. cochlear.

Three stocks of T. trachurus have been identified; one at Bizerte related to L. excisum, one at Kelibia and Mahdia characterized by Cemocotyle trachuri, Prodistomum polonii and Tergestia laticollis and one stock at Zarzis characterized by the absence of P. polonii, L. excisum and T. laticollis.

The variation in the number of ectoparasites and their transmission between regions could be related to environmental factors such as temperature, salinity and density of the host population. Indeed, the northern region, the gulf of Hammamet and the southern region are characterized by different environmental and oceanographic factors. The
variation in the number of endoparasites and their infestation parameters between regions could be related to the distribution and the abundance of intermediate hosts in these areas. Therefore, the diet of a host species in one region may be different from that of other localities. Selected parasites from three small pelagic fishes were used in stock discrimination analyzes proved to be good biological tags to identify and separate stocks of these fishes.

A general framework for an holistic approach to fish stock identification: the example of the HOMSIR project with the Atlantic horse mackerel. (P. Abaunza. IEO)

The EU-funded HOMSIR project was carried out with the aim of obtaining management units that were meaningful biological entities and thus improving the management of the horse mackerel resource. This project started in 2000 and finished in 2004. The results of the project are showed and discussed as an example of a multidisciplinary approach to stock identification.

In the HOMSIR project, the stock identification was made by integrating different approaches such as genetic markers (allozymes, mitochondrial DNA, microsatellite DNA and SSCP on nuclear DNA), morphometry, parasites as biological tags, and life history traits (growth, reproduction and distribution). The sampling covered almost the whole distribution range of horse mackerel through 20 sampling localities in Northeast Atlantic and Mediterranean Sea. Samples were collected during two years and when possible during the spawning season. The sampling size was about 200 specimens per sampling site (with a minimum of 100 specimens per sampling site). All the techniques were applied on the same specimen to reduce the associated uncertainty as much as possible. The holistic approach to stock identification implies the participation of different experts that normally belong to different laboratories and countries. The biological material, therefore, has to be transported from the place where it was collected at sea to the different analysis laboratories. In addition, performing various different techniques on the same fish means that certain criteria need to be taken into account when organizing the sampling process. Thus, the logistic aspect is crucial. Practical procedures must be taken into consideration to ensure consistency in activities and sampling, such as reducing the number of laboratories responsible for providing the biological material to the remaining teams to a minimum. In this way, the number of deliveries of biological material, and consequently, possible losses are reduced.

In summary, all efforts should be made to avoid sampling problems that might render it difficult to provide a useful synthesis in the horse mackerel stock identification studies. The results indicated that horse mackerel showed low levels of genetic differentiation, stable genetic structure over the study time and high levels of genetic variability. However, several approaches (morphometrics and parasites) support the separation between the Atlantic Ocean and the Mediterranean Sea in horse mackerel populations, although the most western Mediterranean area (Alboran Sea) could also be mixed with the Atlantic populations.

In the Northeast Atlantic, various stocks can be distinguished mainly based on morphometrics, parasites and life history traits: a “southern” stock is distributed along the West Atlantic coast of the Iberian Peninsula south to Cape Finisterre (NW Spain); a “western” stock, along the west coast of Europe from Cape Finisterre to Norway and the “North Sea” stock. These results implied the revision of the boundaries of the southern
and western stocks as previously defined. Results also suggested that adult horse mackerel could migrate through different areas following the west coasts in the Northeast Atlantic (i.e. between Celtic Seas and northern North Sea).

Horse mackerel from the Mauritanian coast is distinguished by its high growth rate and high batch fecundity.

Based on the results from morphometric analysis and the use of parasites as biological tags, the horse mackerel population in the Mediterranean Sea is sub-structured into at least three main areas: western, central and eastern Mediterranean.

In this contribution, we have integrated the fundamental findings of different approaches showing that the holistic approach is the appropriate way to identify horse mackerel stocks, on covering multiple aspects of the biology of the species and reducing the type I statistical error in stock identification. As we have seen, some techniques showed differences between areas while others did not (chiefly the genetic techniques). In order to conduct a suitable analysis of the various results, the nature and time scale of the techniques must be taken into account. Moreover, when differences are detected with any technique, always assuming that the correct methodology has been applied, the reasons underlying the differences should be explained. Thus, only heterogeneity and non-homogeneity can be demonstrated.

5. Multi-dimensional identification of stocks: the STOCKMED approach. (F. Fiorentino. IAMC-CNR)

The project STOCKMED: "Stock units: Identification of distinct biological units (stock units) for different fish and shellfish species and among different GFCM-GSA” was a Specific Contract (No 7 (SI2.642234), funded by the Commission of the European Communities, Directorate-General for Fisheries XIV, within the frame MAREA PROJECT with Conisma. The project started in late December 2012 and lasted 18 months (Fiorentino et al., 2015).

On the basis of analysis of spatial variation in life history traits, review of information coming from fishery and biological research works (e.g. biometry, parasites, physical tagging, multiple genetic markers, species mobility, characteristics and duration of embryo and larval phases, growth parameters, etc.), and careful consideration of hypothetical topographic and hydrological boundaries, the STOCKMED project aimed to provide an updated scientific vision on the connectivity and stock boundaries in the Mediterranean. In particular the project aimed to pursue the following challenging objectives: i) to develop a formal methodological framework and to undertake a multidisciplinary identification of distinct fishery/biological units (stock units) for the most relevant demersal and small pelagic species in the Mediterranean, in order to contribute to the improvement of the quality and the reliability of their assessment; ii) to investigate the relationship between the stock units, the characteristics of the main fisheries involved and the GFCM-GSAs system, and propose solutions for different species and fisheries while ensuring consistency with the main current stratifications for data gathering and statistics reporting and iii) to provide an inventory of gaps and suggestions for further investigations.

More than 30 researchers with different expertizes and belonging to seven Scientific Institution (Italian National Research Council - CNR, COISPA Tecnologia e Ricerca, Hellenic Centre of Marine Research, Consorzio Nazionale Interuniversitario per le Scienze del Mare - CONISMA, Centro interuniversitario di Biologia Marina ed
ecologia applicata - CIBM, Instituto Espanol de Oceanografia - IEO, Malta Centre of Fishery Sciences – MCFS), dealing with fishery biology and ecology were involved in the Project.

The area under investigation was that covered by FAO-GFCM GSAs in the northern sector of the Mediterranean, corresponding to GSAs 1, 5, 6, 7, 8, 9, 10, 11, 15, 16, 17, 18, 19, 20, 22, 23, 24, and 25. The following target species were investigated: Merluccius merluccius, Mullus barbatus, M. surmuletus, Pagellus erythrinus, Solea solea, Trachurus trachurus, T. mediterraneus, Engraulis encrasicolus, Sardina pilchardus, Nephrops norvegicus, Aristaeomorpha foliacea, Aristeus antennatus, Parapenaeus longirostris, Octopus vulgaris, Illex coindetii, Eledone cirrhosa, E. moschata, Galeus melastomus, and Lophius budegassa.

In the STOCKMED framework, the goal of identifying the stock units of target species in the Mediterranean can be stated as ‘identification of spatial units homogeneous with respect to multiple descriptors’. To enable this, an original approach combining Geographical Information Systems (GIS), tools of spatial analysis and Multi-Criteria Analysis (MCA) has been proposed. The STOCKMED framework relies upon evaluation of many heterogeneous information layers, where space is used as the unifying descriptor. Therefore a GIS need to be set up to allow for the gathering, visualization and analysis of the maps depicting the thematic descriptors. Since not all descriptors and/or indicators are of equal importance or based on equally reliable information, techniques of MCA are very helpful to evaluate the relative importance (weights) of multiple descriptors and information sources, providing a formal approach to evaluate competing or concordant evidences on stock boundaries. If competing, a prioritization of criteria, based on experts judgement would contribute to solve conflicts and produce a ranking of the alternatives. If concordant, increased confidence in data interpretation would occur based on a consistent picture emerging from several types of data.

The study was based on available data concerning all those domains that are recognized in the literature as determinants for multidimensional identification of stocks. Since these data derive from studies carried out for several different purposes (e.g. analysis of growth, maturity, spatial distribution, etc.), it was necessary to develop an original and appropriate methodology that allowed for data standardization and analysis. In the STOCKMED project eight main criteria were identified as useful to assess the existence of stock units: sea bottom topography, current pattern, biological traits, parasite occurrence, migration and larval drifting routes, genetics, and species distribution and abundance.

In building the STOCKMED framework four main phases were envisaged. Each phase was characterized by the adoption of different combination of “ad hoc” methodological approaches.

Phase 1 - Structuring the problem - Structuring of the problem and the assessment of the current data situation in Mediterranean. Experts from different disciplines worked jointly for elucidating objectives, factors and descriptors relevant to reach the goal, as well as spatial scales to be used in the analysis (Review available data/information, Define relevant thematic descriptors and Define the spatial scale);

Phase 2 - Generating alternative hypotheses of stock units - identification of the putative stock units of target species satisfying a criterion of homogeneity in the biological domain of population parameters from standardized source of information (MEDITs trawl surveys) (Represent MEDITs biological indicators, estimate weights of
biological indicators by Analytical Hierarchy Process - AHP, Perform spatial Constrained Clustering);

Phase 3 - Selecting the most plausible hypotheses of stock units - Selection of most plausible stock configuration and boundaries (Represent data/information for individual thematic descriptors, Estimate weights of thematic descriptors by Non-Structural Fuzzy Decision Support System - NSFDSS, Perform integration of multiple thematic descriptors by the Cohen's Kappa coefficient of agreement, Perform sensitivity analysis by the Stochastic Multi-criteria Acceptability Analysis - SMAA);

Phase 4 - Proposal of stock units - Identify the final proposal for stock units integrating the most plausible configurations with maps of distribution of fishing effort (by fleet segment); compare the areas where new stock units have been identified with the current configuration of GFCM GSAs.

Examples of application of the STOCKMED framework concerning M. merluccius and the final results on stock units identification for the investigated species were presented. Despite the difficulties of integrating in a rigorous analytical procedure different types of information, the methodology developed allowed to identify plausible geographical stock configurations for 14 species out 19 of the investigated species, including the assessment of their uncertainty.

Concerning the information gaps it must be outlined that these configurations derived by data available which were not collected with the specific objective to a multidimensional identification of stock units and their boundaries. Biological information was scant for cephalopods, G. melastomus and L. budegassa. Genetics and parasites had a low availability of information, especially study with significant spatial coverage and fine spatial scale representation. Although in the last years some studies were published, overall specific studies on modelling eggs and larval drift from spawning to nursery areas were still lacking In the Mediterranean. These studies are considered essential to investigate connectivity within and among stock units in a modern meta-population paradigm.

On the basis of the review of available information it was recommended to improve knowledge in the following fields: i) otolith shape and chemical composition, ii) morphometry and meristics, iii) genetic markers at population level, iv) spatial movements and habitat use at different life stages and species, v) larval drift associated with the current pattern, vi) spatial and temporal pattern of oceanographic fronts, vii) spatial localization of fishing effort and catch composition by species.

However the STOCKMED framework allowed to identify stock units and their boundaries by an objective and replicable approach based on integration of multidimensional data set having space as unifying descriptor. The STOCKMED results represented the first identification of stock structure of main target species in the Mediterranean according to an holistic approach. The proposed stock units and boundaries should be re-evaluated as available information increases. To validate the identified stock units and boundaries «ad hoc” projects in which multi-disciplinary data collection is finalized to stock identification purpose should be promoted.

As main results, stock units identification by STOCKMED resulted in a smaller number of entities compared to the current GFCM GSAs frame suggesting the aggregation of units in bigger areas for all the stocks. However in some situations re-distribution of GFCM statistical rectangles in one GSA or another should be adopted. The aggregation of the current GFCM GSAs in larger areas for stock assessment purposes recalls for a better harmonization of the Data Collection also between different Member States.
harmonization should improve sampling certain métier and period and gathering and make available a better knowledge on the spatial distribution of the fleet. The reorganization of stock units in larger areas implies the necessity of using, developing or adapting stock assessment and forecast tools taking into account the different components of the pressure in terms of catches, fishing mortality and effort by fleet and gear on stock units.

6. Discussion about suitable methodologies, data and resources needs to enhance the knowledge on stock units in the Alborán Sea

Participants agreed to adopt the multidisciplinary approach as in the case of the two previous Mediterranean projects (HOMSIR and STOCKMED) for the development of a new research project with the support of CopeMed II and potential partners. The objective of this new project will be to investigate and delineate the spatial structure of sardine and hake populations in the area of Alboran sea and to compare this potential new limits with the current managerial limits of GSAs 1, 3 and 4. The different disciplines to be involved were also agreed: modelling the hydrodynamics of water masses and life stages movements of fish, genetic markers, body and otoliths morphometry, otoliths elemental composition, stable isotopes analysis, life history traits (growth, reproduction and size distribution) and parasites as markers.

Then, participants divided into sub-groups of experts in the different disciplines and worked separately in the elaboration of technical sheets describing the methodology to be applied, the existing resources in terms of equipment, staff and expertise, the potential needs to be covered by the project along with a preliminary rough estimate of cost for a two-year project. These sheets are included in the last pages of Appendix IV.

7. Development of a sub-regional medium term research program for the definition of stocks units of selected species in the Alboran Sea.

The structure of the proposed project entitled: “Transboundary population structure of Sardine and European hake in the Alboran Sea: a multidisciplinary approach” was outlined and agreed to be completed by CopeMed staff in collaboration with experts within the following weeks. The whole project proposal is included as Appendix IV of the present report.

8. Conclusions, recommendations and closure of the meeting

The different contributions presented provided an overview of the current methods used in the delimitation of fish stocks and summarized available information on population of some target species in the Alboran Sea and adjacent Eastern Atlantic and Western Mediterranean waters. The previous experiences in multidisciplinary projects, as explained by their former coordinators provided insights in gaps, potential errors and priorities of research.

The participants from the national scientific institutions CNRDPA, INRH, IEO, INSTM, CNR, and from the Universities of Sfax, Málaga, la Sapienza and Bologna agreed to undertake a multidisciplinary and holistic study aiming at identifying stock units of *Sardina pilchardus* and *Merluccius merluccius* in Alboran Sea and its adjacent waters. The study would have a minimum duration of two years and could involve other scientific partners as necessary.
Upon the approval of the project by the 10th Coordination Committee of CopeMed II, to be held in Algeria in October 2017, the means for its implementation (funds or in-kind) will be sought with donors, national research institutions and regional organizations including the GFCM.

The main research lines adopted are:

- Hydrodynamic connectivity and larval drifting
- Genetic markers
- Body morphometry and meristics
- Otolith shape and elemental composition
- Stable isotope analysis in tissue and in otoliths.
- Parasites as natural markers
- Analyses of abundance, demographics indices, life history traits and patterns of fishing effort.
- Population dynamics simulations

The workshop agreed to transmit the proposal for the research project to the 19th Session of SAC for possible comments and inputs.

CopeMed staff thanked the participants for their contributions and involvement and to the hosting institution, Casa Mediterráneo, for its support and excellent organization. With the commitment to finalize the report and close it by e-mail in the coming weeks before the meeting of the 19th session of SAC, the workshop was closed.
Appendix I

CopeMed II Workshop on methodologies for the identification of stock units in the Alboran Sea
3-6 April 2017, Alicante, Spain

Venue: Casa Mediterráneo. Plaza del arquitecto Miguel López, s/n, Alicante.

Annotated Agenda

Monday 3rd April 19:00

1. Opening session

2. Background and objectives of the workshop (CopeMed II)

Welcome event by the local organizers: Casa Mediterráneo and Spanish authorities.

Social dinner

Tuesday 4th April 9:00 to 18:00

3. Adoption of the agenda and appointment of the Chairperson

4. Overview of current knowledge, historical trends, stock status and uncertainties about stock boundaries of sardin e and hake in Alboran Sea (H. Farrugio. CopeMed II)

5. Hydrological characteristics of Alboran Sea in connection with larval drift.
   o Hydrodynamic connectivity of the Alboran Sea (J. Garcia Lafuente et al.)
   o Explicit implementation of connectivity estimates in population dynamics and assessment frameworks: the European hake in the northwestern Mediterranean (M. Hidalgo)
   o Connectivity between spawning and nursery areas. What is the right spatial scale for our stock assessment?. (F. Fiorentino)

6. Current approaches and methods used to define stock boundaries.
   6.1. Genetic markers
   o General considerations for a genetic approach to the identification of stock units in the Alboran Sea. (C. Johnstone and M. Pérez)
   o Population genetics of hake species along the Moroccan coasts for fishery management purposes. (K. Mokhtar-Jamai)
   o Genomic approaches and targeted genetic markers can identify fine-scale structuring in marine fish populations. (A. Cariani and F. Tinti)

6.2. Morphometry and meristics.
   o Analysis of morphometric variability in Chelidonichys lucerna populations (triglidae) from Algerian coasts. (T. Filali)
   o Discrimination between stocks of sardine in Algerian coast using the number of vertebrae. Bibliography analysis. (K. Ferhani)
- **Biological parameters of small pelagic fish as an indicator of variability (Case of the Moroccan Atlantic).** (F. Wahbi)

**Wednesday 5th April 9:00 to 18:00**

6.3. Life history traits (growth, reproduction, length-at-age, body condition)
- **State of knowledge on fisheries of sardine and hake in the Moroccan Mediterranean sea.** (M.H. Idrissi)
- **Contribution to the identification of the stock structure of Sardina pilchardus along the Tunisian coasts using growth patterns and body shape.** (A. Gaamour)

6.4. Growth marks and elemental composition of otoliths.
- **Contribution to the identification of the stock structure of Sardina pilchardus along the Tunisian coasts, using otolith shape and microchemistry analysis.** (S. Khemiri)
- **Applications of stable isotopes analysis for fish regional stock, spatial segregation and ecosystems connectivity within the Alboran Sea.** (R. Laiz-carrióń et al.)

6.5. Parasites as natural markers.
- **Integrating parasites as biomarkers in the frame of a holistic approach for stock identification of pelagic and demersal Mediterranean Sea fish species.** (S. Mattiucci)
- **Spatial variability of metazoan parasites and evidence for stock discrimination in small pelagic fishes off the coast of Tunisia.** (M. Feki)
- **A general framework for an holistic approach to fish stock identification: the example of the HOMSIR project with the Atlantic horse mackerel.** (P. Abaunza)

7. Multi-dimensional identification of stocks: the STOCKMED approach. (F. Fiorentino)

**Thursday 6th April 9:00 to 18:00**

8. Discussion about suitable methodologies, data and resources needs to enhance the knowledge on stock units in the Alboran Sea.

9. Development of a sub-regional medium term research program for the definition of stocks units of selected species in the Alboran Sea.

10. Conclusions, recommendations and closure of the meeting
Appendix II

CopeMed II Workshop on methodologies for the identification of stock units in the Alboran Sea
3-6 April 2017, Alicante, Spain

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Appendix III

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PROPOSAL FOR A COPEMED II-RESEARCH PROJECT

TRANSBOUNDARY POPULATION STRUCTURE OF SARDINE AND EUROPEAN HAKE IN THE ALBORAN SEA: A MULTIDISCIPLINARY APPROACH
Background and Introduction

The Alboran Sea is the westernmost portion of the Mediterranean Sea, lying between the Iberian Peninsula and the north of Africa. The Strait of Gibraltar, which lies at the west end of the Alboran Sea, connects the Mediterranean with the Atlantic Ocean. The Alboran Sea is a zonal-oriented basin whose main role is to steer the inflow of Atlantic water through the Strait of Gibraltar towards the interior of the Mediterranean. Overall surface circulation consists of a west-to-east flow while a counter current flowing from east to west occurs in the deep waters. The current pattern of surface waters would isolate the ecosystems of its north and south shores from each other or, at least, establish a considerable obstacle for their connectivity in terms of larval drift and movement of organism more in general (García la Fuente et al., 2017). This simple, yet realistic, description guarantees the west-east connectivity, which can be extended to the Gulf of Cadiz in the Atlantic side of the Strait of Gibraltar, a source of biological material for the Alborán Sea. On the other hand, areas off the Spanish coast in the southern GSA 6 and those off Algerian (GSA 4) and Tunisian Coast (GSA 12) could work as sink of organisms born in the Alboran Sea. Such easily understandable hydrodynamic link is behind the reproductive strategies of keystone fish species of the pelagic domain, which take profit of the prevailing surface circulation pattern to connect spawning and nursery grounds, thus assuring higher survival rates.

Considering the oceanographic patterns in the Alboran Sea, many marine living resources may potentially be considered as shared resources in the area (Farruggio, (in prep.)). Sardine and hake are important species exploited in the area by Algerian, Moroccan and Spanish fleets. They belong to the list of “priority species” of the General Fisheries Commission for the Mediterranean and from a management point of view they have been considered as belonging to 4 independent stocks: the northern one, corresponding to the GFCM Geographical Sub-Area 01 (GSA 01: waters off the Spanish coast), the southern one corresponding to the GFCM/GSA 03 (waters off the Moroccan coast), the waters surrounding the island of Alborán (GFCM/GSA 02) and the western part of the GFCM/GSA 04 (Algerian coast).

Figure - 1. Limits of the four GFCM Geographical sub areas in the Alboran sea.

Due to the importance of fisheries in the Alboran Sea, stock identification is a main theme to manage fishery resources in a sustainable way. Stock assessment and fishery management indeed apply population models assuming that the group of individuals have homogeneous vital rates (e.g., growth, maturity, mortality) and a closed life cycles, in which young fish in the group were produced by previous generations within the same group.
In this context, it is evident that the effective definition of the different stocks is a prerequisite for proper data acquisition and subsequent assessment of the state of resources and, consequently, for the formulation and implementation of science based management measures.

Despite its recognized importance, stock identification remains one of the most confusing subjects in fisheries science, with a wide variety of approaches, rapidly advancing methodologies, challenges in sampling, as well as conflicting terminologies and interpretations (Cadriń et al., 2014).

There have been some excellent reviews on stock identification research and numerous definitions of stock are available in literature, with diverse grade of emphasis on phenotypic/environmental, genetics or management aspects (Simon and Larkin, 1972; Ihssen et al., 1981; Templeman, 1982; Kumpf et al., 1987; Pawson and Jennings, 1996; Begg et al., 1999; Waldman, 1999). Two recent review of stock definition are reported in Waldman (2005) and Secor (2014).

Considering the definitions mainly focused on management aspects, Casselman et al. (1981) defined stock as “. . . a population of fish that behaves as a cohesive unit whose members exhibit common responses to environmental conditions within its geographic boundaries”. This definition includes, but is not restricted to population units that are reproductively isolated, which is less restrictive than many other definitions and which conceivably could embrace other concepts such as a regional stock or metapopulation.

A robust, yet sufficiently specific to be useful, definition of stock was offered by Ihssen et al. (1981), who proposed that a stock is “. . . an intraspecific group of randomly mating individuals with temporal or spatial integrity”.

According to Hilborn and Walters (1992) stocks are considered as arbitrary groups of fish large enough to be essentially self-reproducing, with members of each group having similar life history characteristics.

A more recent and operational definition of a stock in fisheries science was given by Begg et al. (1999), according to whom a stock “is a semi-discrete group of specimens of the same species of fish with some definable attributes which are of interest to fishery managers”. These attributes should include spatial distribution, self-renewing properties, size and demographic features, and reaction to environmental factors and fishery pressure.

Despite their variety, all these definitions include the shared occupancy of space as a common factor characterizing stock discreteness. The concept of isolated population with closed migration circuits among spawning, nursery and feeding areas was classically at basis of stock identification (Fig. 2) (Secor, 2002).

![Figure 2 - Migration triangle as depicted by D. H. Cushing where the migration triangle is due mainly to an ontogenetic circuit linked to life cycle (from Secor, 2002).](image-url)
With the increasing importance of spawning stock–recruitment relationship and the improvement of genetic techniques in the last decades, the “stock concept” was refined to include more clearly a degree of reproductive isolation (Booke, 1999). Molecular genetic techniques appeared to be robust tools in conservation biology for identifying reproductive isolation between population, permitting a more effective delineation of management units and allowing assessment of self-maintaining capability of exploited population and sustainability of fisheries (Begg et al., 1999).

Following Waldman (2005) the main characteristics of stocks that are essential for management purposes can be summarized as:

1. stocks occupy their own physical life history circuit, including spawning and nursery areas, that are geographically or temporally discrete;
2. stocks experience their own natural demographic influences, such as mortality suffered from a suite of predators and, more in general, environmental conditions;
3. stocks’ complete-to-partial reproductive isolation allows fine tuning of the morphological and genetic characteristics to the environmental conditions; and
4. stocks abundance and life history characteristics react to the specific anthropogenic pressures to which they are subjected, such as fisheries and habitat contamination/degradation.

In a recent book on stock identification methods, Cadrin et al. (2014) provides an interesting diachronic perspective about studies on stock and fishery management units’ identification. Due to technological improvement in observing population at sea, these studies have changed over time the point of view. The earliest definitions of stock and management units reflected mainly knowledge on fishing grounds. When populations dynamics entered fisheries sciences the role of demography, and the study of vital rates (e.g., growth maturity, recruitment) were emphasized. The subsequent attention on spawning stock-recruitment dynamics led to investigations of life cycle closure and fish movement patterns. With the advances in genetic techniques, the “stock concept” was largely based on reproductive isolation (fig. 3).

Figure 3 – Evolution of concept of stock in fishery science due to improvement of investigation approaches. A progressive change from an emphasis on the fisheries aspect to the reproductive isolation of population was observed (drawn from Cadrin et al., 2014).

A wide spectrum of methodological approaches exist in fishery science for identifying fish stocks. Classically these methods can be based upon: the interpretation of distribution and relative abundance of catch data, tag recoveries, meristics, morphometrics, scale and otoliths morphology and microchemistry, parasites, cyogenetics, protein electrophoresis (isoelectric focusing), immunogenetics, mitochondrial DNA, nuclear DNA, the elemental composition of otoliths, stable isotope measurements, otolith microstructure, and life-history parameters (Beggs & Waldman., 1999; Cadrin et al., 2005). Recent developments in molecular biology, electronic tags, chemical methods, and image analysis have deeply modified the traditional stock identification approaches (Cadrin et al., 2014). New genetic techniques (SNPs) have been
developed and refined. Electronic tagging technology has also rapidly advanced with developments in acoustic, archival, and satellite tags. Additionally, simulation studies, including the larval drift and migration patterns, have emerged as a useful tool for understanding the role of stock structure for fishery management.

However, the acknowledgment that no single method is able to address the various aspects of stock assessment and fishery management, has led to a more holistic view of population structure that requires multiple sources of demographic and genetic data (Pawson & Jennings, 1996; Begg & Waldman, 1999; Cadrin et al., 2005; Cadrin et al., 2014).

Furthermore, recent improvements in knowledge on stock structure of marine organisms challenge the traditional view of populations like geographically distinct units with homogeneous vital rates and isolation from adjacent resources. More complex concepts such as metapopulations may be more applicable to many fishery resources with population structure organised in several sub-units with different level of connectivity (Stephenson, 1999; Kritzler & Sale, 2004).

![Figure 4. Schematic representations of the four types of spatially structured populations. (a) unit population with spatial structure: individuals of the species are unevenly distributed within a single reproductive population; (b) spatial isolation: organisms form a single, self-sustaining biological unit that does not overlap in space with other populations; (c) natal homing: individuals maintain self-sustaining populations through natal homing despite spatial overlap with other populations during certain time periods; (d) metapopulation: multiple, mainly self-sustaining, subpopulations are connected through migration between units, while organisms do not demonstrate spawning site fidelity.](image)

A growing attention to the spatial structure and its connectivity in fisheries sciences will require to clearly identify the stock components, evaluate the movement rates and determining the degree of reproductive isolation. Because spatial structure affects how populations respond to fisheries, incorporation of heterogeneous patterns and movement in stock assessment models should improve advice for fishery management.

In the Mediterranean current stock assessment and fisheries management are highly oriented by the division in Geographical Sub-Areas (GSA) of the GFCM Area. The definition of GFCM-GSA was done on the basis of various criteria and analysis carried out in the early 2000s (oceanographic, biological, fishery, continuity of FAO-GFCM capture statistics, etc.). In the Mediterranean, the knowledge on stock units is still limited and very rough stock boundaries of
main commercial species are available for stock assessment and management purposes. Some recent papers on genetic approaches reported within-species significant genetic differences indicative of reproductively distinct units for some species within the Mediterranean (Tinti et al., 2002; Guarniero et al., 2004; Cimarruta et al., 2005; Garoia et al., 2007; Ramon et al., 2007; Rolland et al., 2007; Maggio et al. 2009; Lo Brutto et al., 2012; Lo Brutto et al., 2013; Milano et al., 2014).

Few papers using other approaches in stock identification are available and concern morphologic/meristics analyses (Mamousis et al., 1988; Orsi Relini et al., 1992; Turan, 2004), morphology joined to micromolecular approaches (Levi et al., 2004), and chemical composition of otoliths (Taller et al., 2013).

A recent review of use of Anisakis larvae in identification of populations units of hake and horse mackerel in the Mediterranean and Atlantic Ocean was due to Mattiucci et al. (2015).

An exercise of identification of putative stock units of European hake, red mullet and deep-water pink shrimp in the Mediterranean Sea, using simple correlation analysis (Pearson’s correlation coefficient) of trends in survey abundance was attempted by Cheilari and Rätz (2009). Bottari et al. (2012) compared abundance data, biological features, growth and mortality parameters of the thornback ray in two adjacent areas of the strait of Sicily and suggested that from a management point of view, the high differences in abundance and the low-intermingling rate between the two areas could support the hypothesis that the investigated populations evolved in two distinct unit stocks.

As regard the holistic approach to stock identification, few examples are available in literature about fishery resources in the Mediterranean. A formal approach by Principal Components Analysis based on different kind of data collected on the same individuals is reported in Cadrin et al., 2014 and concerns the Striped sea bream, Lithognatus mormyrus, in the Mediterranean Sea. An independent population biology study characterized genetic differences in this species, showing the different signals yielded using microsatellites and mitochondrial DNA and revealing that parasitic fauna approximated more closely the structure identified using microsatellites (Sala-Bozano et al., 2009). The analysis of life history data (growth, maturation, sex change) offered additional information, detecting differences between groups that were otherwise indistinguishable (Sala-Bozano and Mariani, 2011). Authors grouped by Principal Components Analysis individual striped sea bream on the basis of 20 different variables (i.e., 14 parasites, microsatellite assignment, mtDNA lineage, weight, length, condition factor, coefficients for growth, maturation, and sex change). The study demonstrates that when all available data for each individual are analyzed in a multivariate framework, it is possible to obtain an overall picture of the relationships among population inhabiting the studied areas, which is more exhaustive than that obtained with any one method employed separately.

Another example of stock units investigation based on a integrated analyses of different source and type of information was given by Quetglas et al. (2012). They presented a view of stock structure concerning the main biological and ecological aspects that should be considered to delineate different management units in the Mediterranean. They focused on the Balearic Islands (GSA05) as case study, highlighting its specificities compared to the adjacent coast of the Iberian Peninsula (GSA06), and suggesting that the approach could be generalized to the problem of identifying stock boundaries in other areas. The work combines the use of published information from different marine disciplines such as geomorphology, ecology and fisheries, with the analysis of new data coming from official fishery statistics and scientific surveys. Such approach circumvents the important drawbacks (inconclusive results,
high costs) of other time-consuming techniques used in stock identification such as genetics. According to the information presented, they concluded that GSA 05 should be maintained as an individualized area for assessment and management purposes in the western Mediterranean. Although the approach used multisource data, the conclusion of analyses was based mostly on comparison of patterns with no formal statistical procedure.

The EU-funded HOMSIR project carried out between 2000 and 2004 aiming at obtaining management units that were meaningful biological entities and thus improving the management of the horse mackerel resource. In the HOMSIR project, the stock identification was made by integrating different approaches such as genetic markers (allozymes, mitochondrial DNA, microsatellite DNA and SSCP on nuclear DNA), morphometry, parasites as biological tags, and life history traits (growth, reproduction and distribution).

The sampling covered almost the whole distribution range of horse mackerel through 20 sampling localities in Northeast Atlantic and Mediterranean Sea. All the techniques were applied on the same specimen to reduce the associated uncertainty as much as possible. The results indicated that horse mackerel showed low levels of genetic differentiation, stable genetic structure over the study time and high levels of genetic variability. However, several approaches (morphometrics and parasites) support the separation between the Atlantic Ocean and the Mediterranean Sea in horse mackerel populations, although the most western Mediterranean area (Alboran Sea) could also be mixed with the Atlantic populations.

Finally, in the framework of an ongoing Italian flag project RITMARE (http://www.ritmare.it/), several studies are being carried out to investigate stock structure of important commercial resources (Engraulis sp., Mullus barbatus, Parapeneus longirostris) applying genetic, parasitic and morphometric approaches to the same individuals.

A first attempt to the identification of the stock units and their boundaries at Mediterranean scale was due to the STOCKMED Project (Fiorentino et al., 2015). The study was based on available data concerning all those domains that are recognized in the literature as determinants for multidimensional identification of stocks. Since these data derive from studies carried out for several different purposes (e.g. analysis of growth, maturity, spatial distribution, etc.), it was necessary to develop an appropriate methodology that allowed for data standardization and analysis. A more detailed description of both projects (HOMSIR and STOCKMED) is reported in pages 15 – 17 of the present report as well as in the full reports of the projects (Abaunza et al., 2008 and Fiorentino et al. 2015)

An example of most plausible stock configuration and their boundaries for Hake is reported in figure 6. On the basis of biological and oceanographical features current GSA were aggregated in larger stock units. There are few cases were the current GSA showed boundaries different from the current ones (GSA 7, GSA 17 and GSA 24).

These configurations represent the first example of the distribution of units of stocks in the Mediterranean according to a holistic - standardized approach and based on the current knowledge available to be validated through specific studies or the collection of supplementary/independent information. Although with some differences in species, results suggested that most of the existing GSA should be aggregated for stock assessment purposes according to a longitudinal gradient. The final results of the application of the STOCKMED framework to the target species is reported in table1.
Table 1. Synoptic table of the identified stock units according the STOCKMED framework. The spatial distribution of the stock units are compared with the current GFMC GSAs.

According to the STOCKMED results populations inhabiting the Alboran sea should be generally included in the stock units living in the western part of the Mediterranean which main border with the central Mediterranean stocks lies in the Gulf of Lions. It is worth noting that the spatial scale under investigation of the STOCKMED project was not the right one to study the stock units distribution in the Alboran Sea that was considered as the border of the Mediterranean Sea.
Some main remarks from the STOCKMED project can be recalled because they can be useful in any stock identification study.

Firstly biological information was scant for cephalopods, black-mouth dogfish and black-bellied angler. Genetics and parasites had a low availability of information (especially study with significant spatial coverage and fine spatial scale representation).

Some main remarks from the STOCKMED project can be recalled because they can be useful in any stock identification study. Although in the last years some studies were published overall specific studies on modelling eggs and larval drift from spawning to nursery areas were still lacking in the Mediterranean. These studies are considered essential to investigate connectivity within and among stock units in a modern meta-population paradigm.

In order to fill some gaps useful for stock identification, it was suggested to improve knowledge in the following fields: i) otolith shape and chemical composition, ii) morphometry and meristics, iii) genetic markers at population level, iv) spatial movements and habitat use at different life stages and species, v) larval drift associated with the current pattern, vi) spatial and temporal pattern of oceanographic fronts, vii) spatial localization of fishing effort and catch composition by species.

Since data used were not collected with the specific objective to stock unit identification, the stock units and boundaries proposed by STOCKMED should be re-evaluated as available information increases. To validate the identified stock units and boundaries «ad hoc» projects in which multi-disciplinary data collection is finalized to stock identification purpose should be promoted (Abaunza et al., 2014).

As main results, stock units identification by STOCKMED resulted in a smaller number of entities compared to the current GFCM GSAs frame suggesting the aggregation of units in bigger areas for all the stocks. However in some situations re-distribution of GFCM statistical rectangles in one GSA or another should be revised.

Based on all these previous studies, and at the request of Scientific Advisory Committee of the GFMC to deepen in the structure of fish populations, the FAO Regional Project CopeMed II, organized the present workshop to propose a specific research program to identify the stock structure and boundaries of Sardine and Hake inhabiting the Alboran sea and the adjacent waters.

The proposal of this new project with details of the methodology by the different project components are reported hereunder.
OVERALL METHODOLOGICAL APPROACH

The proposed project aims:
- To identify the stock units of sardine and hake and their boundaries in the Alboran Sea according a multidisciplinary approach. The study will contribute to better define the appropriate spatial scale for sardine and hake stock assessment and management in the area;

The methodology will follow these steps, (not necessarily in chronological order):
- to examine existing information on the stock structure of sardine and hake in the Alboran Sea and adjacent areas, including upstream the Atlantic Ocean close to the Gibraltar Strait and downstream the portion of the Western Mediterranean more directly affected by the current entering from the Strait of Gibraltar.
- to propose an *ad hoc* sampling program for a minimum of two years in which the same individuals will serve to different type of analysis (*i.e.*: genetics, parasites, morphometry).
- to model the movements of the different life stages of fish coupled with the hydrodynamics of water masses in Alboran area.
- to analyze the information produced with a multidisciplinary and holistic approach.

As reported in the background and introduction section, one of the main themes in the identification of stock structure and boundaries is the strength of interdisciplinary analyses. In the last decades, a lot of methods have been developed and proposed as proper ways to deal with stock identification. However, applying different approaches may lead sometimes to competing hypotheses, whilst a general agreement exists on the need to adopt a holistic approach with multiple perspectives to improve information on stock structure for resource management.

According to Cadrin *et al.* (2014) the process for multidisciplinary identification of the most likely population structure and recommendations for the most appropriate management units requires a review of available information, synthetic conclusions, and practical considerations for management. Moreover, the whole process must adhere to principles of best scientific information available, which should be relevant, inclusive, objective, transparent, timely, verified, validated and peer-reviewed (Cadrin *et al.*, 2014).

Following Cadrin *et al.* (2014), the stock identification process can be summarized in 6 steps

1. A clear definition of the current spatial management units.
2. The identification of all *a priori* hypotheses and paradigms about the population structure, and their internal consistency.
3. Comprehensive information of the specific fishery resource, with emphasis on data and results arising from research explicitly intended for stock assessment.
4. An interdisciplinary evaluation, whose goal is an integrated and holistic knowledge of the life history of the stock.
5. A statistical revision, selecting and using all the information useful for rigorous testing, followed by simulations inside the area and in view of its neighboring connectivity (Kerr and Goethel, 2014).
6. All the necessary recommendations for practical units which reflect not only the stock biology but also other aspects of managing and monitoring its fishery.
Figure 1 - The logical framework in stock identification followed by SIMWG of ICES. (drawn from Cadrin et al., 2014).

Having this conceptual schema in mind and based on the experience gathered in previous studies as analyzed during the “CopeMed II Workshop on methodologies for the identification of stock units in the Alboran Sea” (Alicante, Spain 3-6 April 2017) a proposal for a new research project was drafted which is summarized below. The project will address sardine and hake stocks in the Alboran Sea. The structure follows a series of phases to be developed in a period of two years, extendable according to the funds made available by CopeMed II donors and partners.

The 4 existing GSAs in the Alboran Sea will represent the “a priori” stock hypothesis to be tested. Information coming from single disciplines will be collected and scrutinized with the objective to be analysed by an holistic approach similar to the framework proposed by the STOCKMED project. Differently from the STOCKMED project that was based only on existing data, an ad hoc sampling scheme will be designed to collect new data and samples. Sampling will be designed by the experts on an initial phase with the purpose to cover the spatial and temporal scales of the life cycle of the two-species concerned. The same individuals will provide biological material for the different analysis. The disciplines involved are: hydrodynamic connectivity, genetic markers, parasites as markers, otolith shape and elemental composition, body morphometry and meristics, analyses of fishery patterns, demographics indices and life history traits.

The main phases of the project are outlined in the section below. The last section of this document: Project Components includes technical sheets produced by different groups of experts in the different disciplines during the CopeMed II workshop and represent the preliminary description of methods and needed resources in a more detailed way.

The theoretical spatial concept of the project can be represented in figure 2.
Figure 2: the source-sink dynamics of a species during its life cycle in an area and its adjacent upstream and downstream vicinities. S: spawning area; N: nursery area; red arrows: adults displacements; black arrows: larvae displacement towards the nursery areas. (from Fiorentino et al., in prep.)

PHASES OF THE PROJECT:

Modelling
A hydrodynamical-biological model describing the movements of simulated particles in the Alboran sea will be used to simulate the dispersal and/or retention of eggs and larvae and examine both potential and effective connectivity between the northern and southern continental shelves of the Alboran basin and the vicinities. Metrics of connectivity will be calculated for defined areas and periods that reproduce the spawning to the recruitment of each species and the outputs will be compared with observations from surveys and landings. A series of years of data will be selected per species including contrasting years of high and low abundance as well as contrasting oceanographic conditions to test the model outputs.

Sampling
Experts from different disciplines will work jointly according to a determined sampling strategy that will cover the different spatial and temporal scales needed for each analysis. The necessary number of fish will be collected during the regular sampling programs (surveys and landings) in the four countries: Algeria, Morocco, Spain and North Tunisia. The biological material will be processed and analysed, or sent to the laboratories responsible for the different analysis: genetic markers, parasites, otoliths shape and elemental composition, stable isotopes, body shape and meristics of hard structures.

Information Processing

- Spatial analysis

The information produced in the steps above together with all data compiled referred to the fisheries indicators, biological parameters and demographic and spatial metrics will be collated and processed with integrative statistical tools.
— Multivariate statistics:

Multivariate statistical techniques as the Multi Criteria Decision Analysis (MCDA) will be used at a later extent. These techniques include: selection of best descriptors, assignment of weights by Non-Structural Fuzzy Decision Support System - NSFDSS, perform integration of multiple thematic descriptors by the Cohen's Kappa coefficient of agreement, perform sensitivity analysis by the Stochastic Multi-criteria Acceptability Analysis -SMAC-. This exercise of synthesis will help in the elaboration of alternative hypothesis of stock boundaries.

Proposal of stock units
The final proposal of new possible configurations of stock units will be integrated with maps of distribution of fishing effort (by fleet segment). The areas where new stock units have been identified will be compared with the current configuration of GFCM GSAs.

The final aim is to describe the spatial distribution of the resources in the most precise way to be applied in the regular assessments and to inform the management advice for sardine and hake in Western Mediterranean.
The Project Components

The study area will cover the Alboran sea and adjacent waters including the Atlantic Ocean and southern Mediterranean coast up to northern Tunisia trying to cover the whole distribution area of the two species selected (sardine and hake) with the aim to better understand the movements of fish during all their life cycle. Samples will be taken from a set of locations well distributed along the whole study area in periods that cover the different life stages. The same specimens will serve to take different measures, otoliths and tissue samples for different analysis: genetic, elemental composition, isotopes and parasites. In addition, time series from fishery and surveys data will be incorporated.

Scientific teams have been created to oversee each one of the project components (or research lines) established. The outlines of these research lines and resources existing and needed are summarised below:

1. HYDRODYNAMIC CONNECTIVITY

This group will work on the description of the hydrodynamics features that shape the connectivity between spawning and nursery areas in Alborán and its source and sink outer areas. The hypothesis generated by this model will be validated by other methods.

The study will be based on a hydrodynamical-biological coupled model (ocean physics plus lower trophic ecosystem component) that will cover both Gulf of Cadiz and Alboran Sea (until the Gulf of Vera in the north and 1° East in the south) (Figure 1). At temporal scale, the model will solve from tidal to inter-annual scales. This work is already well advanced by the Physical Oceanography group of the University of Málaga can simulate the drifting of fish larvae in both space and time through lagrangian drifter experiments. The experiments will be conducted during the spawning season of the species. Phytoplankton, zooplankton and sea water temperatures, which are two variables provided by the model, will be used to further assess the probability of larvae survival along their drift (e.g. starvation mortality). The model has a mean spatial resolution of 1.5 km in the Alboran Sea, which enables for analysis of sets of cells up to the 30’x30’ statistical grid.

Figure 1. Geographic domain of the hydrodynamical-biological coupled model.
Demographic and size information from acoustic (sardine) and trawling (hake) surveys and landings will be collected for as many years as available in the most recent period.

Samples of larvae and adults (for sardine) and recruits (for hake) will be collected for two consecutive years: 2018 and 2019

**Existing resources**
The University of Malaga (UMA) will use its supercomputing resources for the model simulations, other instruments for physical measurements could be used if required. IEO will collect samples from the different sampling sites during its current surveys and will perform isotope analyses of organic tissues and otoliths.

**Methods and needs.**
The bio-physical modeling will have two steps:

1. Selection of four years per species with enough contrast of high and low abundance, but also contrasting in terms of the general oceanographic scenario.
2. Estimation of connectivity metrics for defined areas and periods that simulate the spawning to the recruitment tracks of each species. The outputs will be compared with observations from surveys and landings.

In addition, analyses of two stable isotopes: bulk-SIA and CSIA will be combined to estimate the ecological segregation in diet and spatial distribution. *S. pilcardus* early life stages (post-larvae) will be collected in the nursery areas from the Gulf of Cadiz and North and South of Alboran Sea. Isotopic values of sardine post-larvae will be related to their respective baseline isoscape signatures (two zooplankton size fractions as putative food sources). Juveniles and/or adults sardines coming from the whole study area will be sampled for otolith SIA and CSIA analyses for retrospective studies of diet and movement across isoscapes. For *M. merluccius*, bulk-SIA and CSIA from tissue and otoliths will be combined in juveniles and adults coming from the whole study area. These outputs together with demographic information from surveys and assessment outputs will contribute to validate the bio-physical model outputs.

**Participants Institutions and coordinator(s)**
IEO Málaga, Baleares and University of Málaga (UMA) from Spain; INRH (Morocco); CNRDPA (Algeria), INSTM (Tunisia) and collaboration of scientists from CNR-IAMC (Italy)
Coordinator of Modeling of larvae dispersal and survival: Manuel Hidalgo IEO Baleares Coordinator of Isotope analysis: Raúl Laiz IEO Málaga
Laboratory assistants are needed.
2. GENETIC MARKERS

Fish will be collected at specific sites from the Alboran Sea, as well as from neighbouring Atlantic Ocean (Spain and Morocco) and neighbouring Mediterranean Sea up to the Northern coast of Tunisia. Specimens must be both, spawners from adult high concentration areas and early life specimens to investigate overlap with boundaries defined by adults, and connectivity with spawning and nursery grounds (source-sink concept from spawning to nursery areas).

Specimens collected in compatible spatial and temporal scales by the different teams (minimum of 40 individuals per sampling site, however higher number of individuals are desirable in order to optionally increase the total sample size if required once genetic analysis are ongoing). A small portion of tissue should be stored in non-denatured ethanol (96%) and transmitted to the laboratories in charge of the genetic analysis. Approximately 33 samples have been taken into account to estimate the cost of the activities; however, the sampling strategy should be designed by experts according to currently undergoing surveys and data collection programs.

Existing resources
INRH: Molecular biology laboratory
University of Bologna (UniBo): Molecular biology laboratory.
IEO: Molecular biology laboratory at Aquaculture facilities in Vigo and in Málaga

The microsatellite laboratory experiments on *M. merluccius* are already underway at INRH (including samples from the Alboran and the Atlantic Ocean). All the information related to *M. merluccius* SNPs are legacy of FishPopTrace Project and can be transferred to any laboratory. *M. merluccius* microsatellite genotyping is ongoing at IEO, Vigo, for aquaculture applications. *S. pilchardus* microsatellite genotyping was carried out in UniBo in the past years.

Method and needs.
*M. merluccius*:
Neutral microsatellite loci (from bibliography and EST-derived possibly non-neutral) (15 loci)
Non-neutral (outlier) SNPs (48 loci)

*S. pilchardus*
Neutral microsatellite loci (15 loci)
SNPs development for Sardina pilchardus is ongoing

Participants Institutions and coordinator(s)
Participants Institutions: IEO Málaga, Vigo (Spain); INRH Nador, Casablanca (Morocco); CNRDPA (Algeria); INSTM (Tunisa); University of Bologna (Italy) and University of Vigo (Spain).

Coordinators of analysis: IEO: C. Johnstone, INRH: K. Mokhtar-Jamaï and UniBo: A. Cariani
Laboratory assistants are needed.
3. PARASITES AS MARKERS

The parasite based methodology (including parasite community structure of the fish species along its geographical distribution, parasitic infection levels and genetic/molecular characterization of parasite species) represents an important approach in defining a fish stock. Indeed a parasite can be used as a suitable biological tag for fish stock identification when its geographical distribution and life cycle are known, and when the parasite’s residence time in the host is long enough, compared with the lifespan of the fish host.

Sampling sites (a minimum of 8) will be selected in agreement with the other groups to make sure that the totality of the study area will be covered. Samples must be obtained during the peak of spawning season which for sardine is in winter and for hake is in Spring.

Samples must contain adults. Preferred larger sizes of both species, e.g. sardine > 160 mm and hake > 400 mm. Estimated numbers of individuals are 50 specimens per sampling site and year of sardine and 35 specimens of hake per sampling site and year.

Existing resources
La Sapienza University can provide infrastructure for genetics of parasites. The samples will be provided by the other institutions following a protocol well established for the preservation of the material. Previous experience in hake and sardine will be included as a comparative analysis. There are research surveys through the study area that can be used to obtain the samples.

Method and needs.
Traditional parasitological analysis including UV-press system; traditional morphological analysis of some parasite species; genetic / molecular identification of anisakids and other nematodes; population genetic analysis of some parasitic species. Statistical analysis of the genetic data and epidemiological parameters including simple and multivariate methods. Comparison with the results from other methods.

Participants Institutions and coordinator(s)
IEO (Spain); INRH (Morocco); CNRDP (Algeria), INSTM (Tunisa) and Sapienza-University of Rome, Italy and Faculty of Sciences Sfax University of Tunisia.
Coordinator of parasites identification: Simonetta Mattiucci from La Sapienza University (Italy)
Coordinator of sampling and analysis of data: Pablo Abaunza from IEO, Madrid (Spain)
Laboratory assistants are needed
Experts from the Faculty of Science (Tunisia) will be in close contact, including short-term visits for the molecular identification, with the University La Sapienza (Italy).
4. OTOLITH SHAPE AND ELEMENTAL COMPOSITION

Otolith shape and microchemistry analysis are useful techniques for discrimination of populations. The chemical composition of whole otoliths is analyzed using solution-based inductively coupled plasma mass spectrometry (ICPMS). In addition, the otoliths shape is analyzed using Fourier analysis. A minimum of 8 sampling sites will be selected in agreement with the other groups to make sure that the totality of the study area will be covered. For each species 120 specimens are needed (length range: 11 to 16 cm for sardine; 25 to 35 cm for hake): 100 for shape analysis and 20 for microchemistry.

Existing resources:
All the equipment for image acquisition of otoliths is available at the IEO, INRH and CNRDPA furthermore, the software needed for the otoliths shape analysis is available at the INSTM.

Method and needs.
Otolith Shape: High-contrast images of complete otoliths will be acquired using reflected light on a black background. A camera linked to a computer will be used to acquire digital images. The shape of each otolith will be assessed with the elliptic Fourier analysis.

Otolith microchemistry: Upon extraction, sagittal otoliths are cleaned in a laminar flow hood. Whole otolith analysis is done by solution-based inductively coupled plasma mass spectrometry (ICP-MS) to permit simultaneous measurement of the concentrations of many elements that are useful for stock identification. Once the otoliths are cleaned and preserved, will be sent to an external laboratory for the ICP-MS.

Participants Institutions and coordinator(s)
IEO (Spain), INRH (Morocco), CNRDPA (Algeria) and INSTM (Tunisia)
Coordinator for otoliths shape and analysis: S. Khemiri from INSTM
5. BODY MORPHOMETRY AND MERISTICS

Morphometric and meristic characters will be used to investigate fish populations. A minimum of 8 sampling sites will be selected in agreement with the other groups to make sure that the totality of the study area will be covered. Samples will be taken from Alboran Sea and adjacent areas: Spain, Morocco, Tunisia and Algeria, from survey and landings, with a frequency of twice a year and out of the reproduction period (between April and September for all countries). Sample size will be up to 50 specimens per station.

Existing resources:
Basic equipment and expertise exist in the four centres. Complementary basic equipment and technical assistance for the analysis of data can be needed.

Method and needs.
Morphometry of body including measurements of some body dimensions will be done with a calliper and through image analysis from pictures. Meristic characters such as the number of vertebrae and branchiopines in dry material will be used to investigate fish populations variability.

Participants Institutions and coordinator(s)
A coordinator will be nominated in each country for the morphometry analysis IEO (Spain), INRH (Morocco), CNRDP (Algeria) and INSTM (Tunisia)
Coordinator for meristics: T. Filali, CNRDP (Algeria)
Assistance of expert in data analysis is needed.

6. ANALYSES OF FISHERY PATTERNS, DEMOGRAPHICS INDICES AND LIFE HISTORY TRAITS.

Existing data and published Information on the biology and fisheries of the two species in the region will be compiled and analysed. The progress on this type of analysis currently done at the Working Groups of stock assessment of the CopeMed II Project will be taken as a basis. Update will be ensured by the partners laboratories who will provide the annual data from surveys and commercial fisheries.

Existing resources:
Information and expertise exist in the four centres. Complementary technical assistance for some specific analysis can be needed.

Method and needs.
Pattern of biomass in space and time, Identification of Essential Fish Habitats of the two target species, Analysis of life traits variability in time and space; Pattern of fishing effort in space and time, Statistical framework for identification of stock units and their boundaries by a multi-dimensional approach.

Scientists from the various national institutions will be in close contact to exchange data sets including one or two working groups to process the data.
Participants Institutions and coordinator(s)
IEO (Spain); INRH (Morocco); CNRDPA (Algeria), INSTM (Tunisa) and collaboration of scientists from CNR-IAMC (Italy)
Coordinators for the compilation of information: A. Giráldez (IEO), MH. Idrissi (INRH)

7. POPULATION DYNAMICS SIMULATIONS

Under the different scenarios of stock discrimination obtained and before formulating any type of recommendation, population dynamics simulations will be carried out to evaluate the consequences of the different assumptions about stock boundaries to the assessment of the resource status and the outcomes of management strategies. This component will be further developed based on the outcomes of the previous components. It is envisaged the use of methodologies for management strategy evaluation.

Coordinator: M. Hidalgo (IEO), Spain
References


